

IN THE CLAIMS:

Please amend claims 1, 2, 18, 22, 24, 35, 39, 41, 42 and 44, as follows:

Sub 1.1
1(Amended). A polynucleotide which, upon in vivo introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, comprising:

a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron;

a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter;

optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter; and

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a transcriptional terminator following each of the first, second and third cistron, unless followed by another cistron lacking its own transcriptional promoter.

2(Amended) The polynucleotide of Claim 1 wherein the first cistron encodes at least one immunogenic epitope of a pathogen [or a cancer associated pathogen].

CA
18(Amended). A polynucleotide which comprises contiguous nucleic acid sequences which cannot replicate in eukaryotic cells in vivo but which are capable of being expressed to produce a gene product upon introduction of the polynucleotide into eukaryotic tissues in vivo, wherein the gene product either acts as an immunostimulant or as an antigen capable of generating an immune response, wherein the nucleic acid sequences encode:

- PK
a) a spliced REV gene;
b) a spliced human immunodeficiency virus (HIV) immunogenic epitope; and,
c) optionally, a cytokine or a T-cell recognition element.

22(Amended). A polynucleotide comprising a first gene encoding an HIV gag, gag-protease, or env immunogenic epitope, the gene containing a REV responsive element (RRE) or having been modified to contain an RRE, the gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a gene encoding a REV gene product, wherein said polynucleotide is non-replicating in eukaryotic cells in vivo.

24(Amended). A polynucleotide which is non-replicating in eukaryotic cells in vivo and induces anti-HIV neutralizing antibody, HIV specific T-cell immune responses, or protective immune responses upon introduction into vertebrate tissue, including human tissue *in vivo*, wherein the polynucleotide comprises a gene encoding a gene product selected from HIV gag, HIV gag-protease, and HIV env, the gene containing a REV responsive element (RRE), the gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene, the second gene encoding a REV gene product.

35(Amended). A polynucleotide which is non-replicating in eukaryotic cells in vivo, comprising:

- a) an eukaryotic transcriptional promoter;
- b) an open reading frame 3' to the transcriptional promoter encoding an immunogenic HIV epitope wherein the open reading frame has a splice donor sequence at the 5'-side of the open reading frame, a REV responsive element anywhere within the open reading frame, and a stop codon encoding the termination of translation of the open reading frame;
- c) an internal ribosome entry site (IRES) 3' to the translation stop codon of the open reading frame;
- d) an open reading frame encoding a spliced HIV REV gene at the 3' end of which is a translation stop codon;

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cont.

e) optionally, 3' to the REV translation stop codon, a second IRES, followed by an open reading frame encoding immunomodulatory or immunostimulatory genes, the genes being selected from GM-CSF, IL-12, interferon, and a B7 protein;

f) a transcription-termination signal following the last open reading frames.

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39(Amended). A polynucleotide which is non-replicating in eukaryotic cells in vivo, [comprises] comprising sequences encoding:

a) an eukaryotic transcription initiation signal;

b) an HIV gene open reading frame (ORF) preceded by an heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;

c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the HIV ORF;

d) a sequence encoding an ORF of a T-cell costimulatory element 3' to the IRES; and

e) a transcription termination signal 3' to the translation stop codon of the T-cell costimulatory element.

C7
B7

41(Amended). A polynucleotide which is non-replicating in eukaryotic cells in vivo, [comprises] comprising sequences encoding:

a) an eukaryotic transcription initiation signal;

b) an HIV gene open reading frame (ORF) preceded by an heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;

c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the HIV ORF;

d) an HIV gene open reading frame (ORF) preceded by an heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product; and

e) a transcription termination signal 3' to the translation stop codon of the HIV gene ORF.

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42(Amended). A composition comprising multiple expression constructs of claim 1, each of which is capable of inducing expression in mammalian tissue of more than a single cistron encoding antigens related to disease causing pathogens or tumors.

Sub
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44(Amended). A polynucleotide construct which is non-replicating in eukaryotic cells in vivo, having the elements shown in figure 2, wherein each of the first, second and third cistrons shown in the figure encode a combination of any two to three of the following:

- 1) tPA-gp120MN;
- 2) gp160IIIIB/IRES/REVIIIIB;
- 3) gp160IIIIB;
- 4) REVIIIIB;
- 5) tat/REV/gp160;
- 6) REV/gp160;
- 7) gp160MN;
- 8) gp160 from clinically relevant primary HIV isolates;
- 9) nef, using the gene from clinically relevant strains;
- 10) gagIIIIB;
- 11) tPA-gp120IIIIB;
- 12) gp160 with structural mutations including V3 loop substitutions from clinically relevant strains of HIV; several mutations on several constructs such as variable loop removal, Asn mutations to remove steric carbohydrate site obstacles to structural, neutralizing antibody epitopes; and CD4 binding knockout mutants;
- 13) gp41 with provision of appropriate leader sequences, as in the tPA signal peptide leader sequence;
- 14) gag: similar to construct from #5 above, using the gene from clinically relevant strains;
- 15) rev: for gp160 and gag dicistronics;
- 16) B7 coding sequences;
- 17) GM-CSF sequences;
- 18) Interleukin sequences;
- 19) Tumor associated antigens;